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*Abbreviations:* gas chromatography/mass spectrometry (GC/MS), high pressure liquid chromatography (HPLC), dihydrotestosterone (DHT), hypothalamic-pituitary-gonadal (HPG), single ion monitoring (SIM).

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## Abstract

Little is known concerning the potential ecological effects of hormonally active substances associated with discharges from animal feeding operations. Trenbolone acetate is a synthetic anabolic steroid that is widely used in the USA to promote growth of beef cattle. Metabolites of trenbolone acetate include the stereoisomers  $17\alpha$ - and  $17\beta$ -trenbolone, both of which are stable in animal wastes and are relatively potent androgens in fish and mammals. The purpose of this study was to evaluate the occurrence of  $17\alpha$ - and  $17\beta$ -trenbolone in a beef cattle feedlot discharge and in river water upstream and downstream from the discharge. In conjunction with this, we measured *in vitro* androgenic activity of the discharge using CV-1 cells that had been transiently cotransfected with human androgen receptor and reporter gene constructs. Samples were collected on nine different occasions during 2002 and 2003. Whole water samples from the discharge caused a significant androgenic response in the CV-1 cells and contained detectable concentrations of  $17\alpha$ - and  $17\beta$ -trenbolone. Further work is needed to ascertain the degree to which synthetic androgens such as trenbolone contribute to androgenic activity of feedlot discharges.